

## A LATITUDINAL CLINE OF DARK PLASTRAL PIGMENTATION IN THE TORTOISE *TESTUDO HERMANNI* IN GREECE

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The area of dark pigmentation on the plastron of the tortoise *Testudo hermanni* shows a latitudinal cline over about 400 km in Greece, with populations in the south being darker. The carapace did not show the clinal trend, and pigmentation was not significantly related to longitude or altitude. We examined several possible explanations for the cline, including an effect of incubation temperature, random genetic variation, and adaptation to several environmental variables. The most likely explanation is selection for thermoregulation, with decreased dark pigmentation in the north reducing heat loss to the substrate by infra red radiation during activity. This hypothesis was supported by data on body ( $T_b$ ) and substrate ( $T_s$ ) temperatures in populations from northern, central and southern Greece.  $T_b$  was generally above  $T_s$ , showing that heat would generally be lost rather than gained through the plastron, and the mean difference  $T_b - T_s$  was greatest in the north: +6.6 °C, compared to +2.4 °C in the south. Mean  $T_b$  was lowest in the south (26.9°C, compared to 29.3°C in the north) and the slope of  $T_b$  on  $T_s$  was about 1 (compared to 0.5 in the north). Thermoregulation in southern Greece is similar to that of tropical tortoises, with avoidance of overheating being the major problem, rather than elevation of  $T_b$  for activity.

*Key words:* cline, pigmentation, *Testudo*, thermoregulation, tortoise

### INTRODUCTION

Many species of reptiles show marked variation in colour pattern within and among populations, particularly in the level of dark pigmentation (Crisp, Cook & Hereward, 1979). Genetic differences in pigmentation may be the result of selection for camouflage (Gibbons & Lillywhite, 1981), reptiles on darker soils often being darker coloured (Lewis, 1949; Lawrence & Wilhoft, 1958). Dark pigmentation also has consequences for thermoregulation, melanistic individuals reaching higher body temperatures than normally pigmented individuals (Gibson & Falls, 1979). Dark pigmentation may also be involved in protecting the tissues from damaging ultraviolet (UV) radiation (Porter & Norris, 1969; Cloudsley-Thompson, Constantinou & Butt, 1985). Pigmentation may, however, be directly affected by incubation temperature in reptiles (Ewert, 1979; Murray, Deeming & Ferguson, 1990), so that differences among populations are not necessarily genetic.

There is considerable variation in the extent of dark pigmentation on the plastron of the Mediterranean tortoise *Testudo hermanni*, particularly between the western (*T. h. hermanni*) and eastern (*T. h. boettgeri*) subspecies (Guyot & Devaux, 1997). Differences of coloration are of taxonomic significance in some chelonians (Fritz, 1992). The plastron of tortoises is rarely exposed, and is therefore a good candidate for a selectively neutral characteristic of value in differentiating between the subspecies of *T. hermanni*.

Nevertheless, plastral pigmentation varies substantially between populations of *T. h. boettgeri* in Greece; animals from the south have notably dark plastrons, similar to those from western Europe. This paper describes the variation in dark plastral pigmentation of *T. hermanni* in Greece, in relation to geographic and environmental variables. Several hypotheses to explain this variation are then evaluated, in particular that this is due to thermoregulation; data on body and substrate temperatures are used to assess the direction and relative rate of heat exchange through the plastron in different populations.

### METHODS

Pigmentation of *T. hermanni* was studied at 16 sites in Greece (Fig. 1), which are described by Willemsen & Hailey (1989). Tortoises were marked in the field with unique codes by filing the marginal scutes (Stubbs *et al.*, 1984); each pattern considered here is from a different individual. Straight carapace length was measured with a specially-constructed flat-bed calliper or 'tortometer'. Sex was determined on the basis of external characteristics: *T. hermanni* can be sexed from a straight carapace length of 10 cm (Stubbs *et al.*, 1984), and only animals larger than 10 cm are considered here. The pattern of pigmentation was recorded by photographing the carapace and/or plastron as colour slides.

Slides were projected on to squared paper and the area of dark pigmentation was traced out. The area was measured on one side of the plastron and on the costal scutes of one side of the carapace. Dark pigmentation occurs in characteristic areas of the plastron and carapace (typical patterns of dark plastral pigmentation in *T. hermanni* are shown in Fig. 3 of Guyot & Devaux,

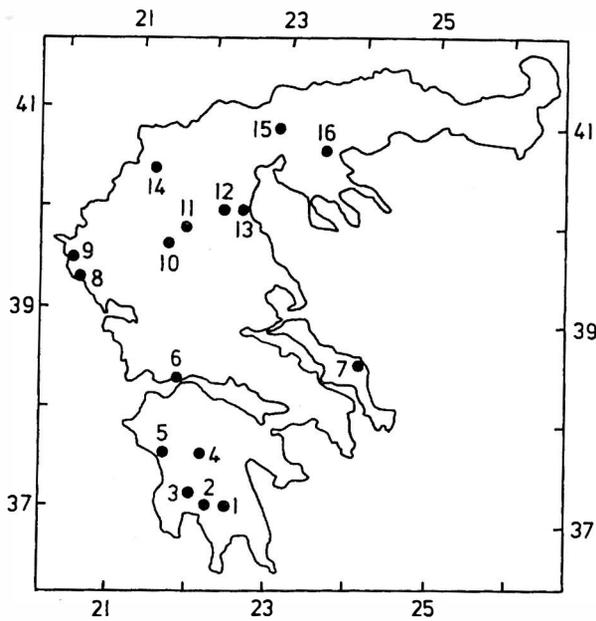


FIG. 1. Map of Greece showing the locations of the sites, and latitude ( $^{\circ}$ N) and longitude ( $^{\circ}$ E). Sites are: 1, Sparta; 2, Kalamata; 3, Arfai; 4, Langadia; 5, Olympia; 6, Antirrhion; 7, Kymi; 8, Parga; 9, Igoumenitsa; 10, Meteora; 11, Deskati; 12, Agios Dimitrios; 13, Litochoron; 14, Kastoria; 15, Kilkis; and 16, Mikra Volvi.

1997), and the pattern is approximately bilaterally symmetrical in each individual. The area of pigmentation was then calculated as a percentage of the total area of the half-plastron or costal scutes, respectively. The arcsine transformation (Sokal & Rohlf, 1981) was used for multivariate analysis of percentage data (but not for calculating means and SDs).

The primary environmental data for each site comprised its latitude, longitude and altitude. The

environmental temperature at each site was summarised as the effective temperature (ET; Stuckenberg, 1969), calculated as  $ET = (8\bar{T} + 14T_r) / (T_r + 8)$ , where  $\bar{T}$  is the mean annual temperature and  $T_r$  is the annual range of temperature. Mean temperature in each month was calculated as (mean daily maximum + mean daily minimum)/2 (Meteorological Office, 1996, page viii);  $\bar{T}$  is the mean temperature averaged across all months, and  $T_r$  is the difference between the means of the warmest and coldest months. A multiple regression showed that ET in Greece and the Balkans south of  $41^{\circ}$ N was significantly related to latitude ( $P < 0.001$ ) and altitude ( $P < 0.001$ ), but not to longitude ( $P = 0.066$ ) (Willemssen & Hailey, 1999). A multiple regression of the significant variables explained most ( $r^2 = 88.3\%$ ) of the variation of ET from latitude and altitude, and was used to predict ET at the tortoise sites.

Substrate temperatures ( $T_s$ ) were measured, simultaneously with body temperatures ( $T_b$ ) of active tortoises, at three sites of similar altitude (200–300 m) in northern, central and southern Greece. Data from Mikra Volvi were collected from 17–20 April 1989. More data were available for the other two sites, but only those collected at about the same time are considered here: 24 April – 17 May 1989 at Meteora and 20–23 May 1989 at Sparta. Active tortoises were measured at the time and place of capture, throughout the daily activity period. Temperatures were measured with a mercury thermometer to the nearest  $1^{\circ}$ C:  $T_b$  in the cloaca, and  $T_s$  at the surface near the tortoise.

## RESULTS

### INDIVIDUAL AND SEXUAL VARIATION

There was continuous variation in the area of dark plastral pigmentation between individuals within all

TABLE 1. Altitude, effective temperature (ET) and pigmentation of *T. hermanni* at different sites. Values are the mean  $\pm$  SD area of dark pigmentation as a percentage of the area of plastron or costal scutes of the carapace, with sample size in parentheses.

	Altitude (m)	ET ( $^{\circ}$ C)	Area dark (%)	
			Plastron	Carapace
Sparta	300	14.9	56.0 $\pm$ 14.6 (156)	41.6 $\pm$ 14.2 (112)
Kalamata	0	15.4	60.2 $\pm$ 19.1 (77)	35.4 $\pm$ 11.2 (69)
Arfai	150	15.1	60.2 $\pm$ 16.3 (7)	38.7 $\pm$ 7.4 (8)
Langadia	1250	13.0	39.3 $\pm$ 13.8 (47)	44.3 $\pm$ 13.6 (44)
Olympia	200	15.0	51.3 $\pm$ 16.2 (120)	42.4 $\pm$ 14.7 (171)
Antirrhion	50	15.0	37.4 $\pm$ 7.3 (8)	34.7 $\pm$ 13.9 (7)
Kymi	150	14.8	41.9 $\pm$ 10.1 (12)	50.9 $\pm$ 11.1 (8)
Parga	300	14.3	29.7 $\pm$ 8.1 (38)	49.7 $\pm$ 11.1 (35)
Igoumenitsa	0	14.8	36.6 $\pm$ 12.7 (104)	46.9 $\pm$ 14.4 (93)
Meteora	250	14.3	26.3 $\pm$ 15.7 (130)	38.5 $\pm$ 16.8 (187)
Deskati	650	13.6	23.6 $\pm$ 13.1 (116)	32.8 $\pm$ 16.4 (108)
Agios Dimitrios	600	13.6	22.1 $\pm$ 14.2 (89)	35.5 $\pm$ 19.8 (73)
Litochoron	450	13.9	34.6 $\pm$ 17.5 (49)	49.5 $\pm$ 15.4 (49)
Kastoria	800	13.1	27.4 $\pm$ 10.6 (100)	39.0 $\pm$ 14.4 (94)
Kilkis	250	14.0	22.5 $\pm$ 9.8 (45)	24.8 $\pm$ 11.4 (40)
Mikra Volvi	200	14.2	21.8 $\pm$ 13.4 (34)	36.7 $\pm$ 16.1 (34)

populations, with no evidence of separate classes (i.e. polymorphism); examples of two populations from northern and southern Greece are shown in Fig. 2. There was a significant tendency for males to have a larger area of dark plastral pigmentation than females (Fig. 3). The regression equation is:  $M = -4.0 + 1.30F$  ( $n = 16$ ,  $r^2 = 91.4\%$ ), where  $M$  and  $F$  are the mean areas of dark plastral pigmentation for males and females, respectively. The slope is significantly different from 1.0 ( $t = 2.77$ ,  $P < 0.02$ ), but the intercept is not significantly different from 0 ( $t = 1.07$ ,  $P = 0.303$ ). The area of dark plastral pigmentation was thus about 1.3 times greater in males than in females, across all populations.

The effect of body size on plastral pigmentation was examined separately in males and females, as there is sexual size dimorphism in Greek populations of *T.*

*hermanni* (females being larger; Willemsen & Hailey, 1999). The correlation coefficient between carapace length and the area of dark pigmentation was calculated for all samples with 30 or more individuals. The mean value of  $r$  was  $-0.020$  ( $SE = 0.043$ ,  $n = 15$ ), not significantly different from 0 ( $t = 0.46$ ,  $P = 0.65$ ). There was thus no consistent effect of body size on the area of dark plastral pigmentation among these populations.

The final question on variation within populations concerns the independence of pigmentation on the plastron and carapace. The correlation coefficient between the area of dark pigmentation on the plastron and on the carapace was calculated for all samples with 30 or more individuals with both measurements. The mean value of  $r$  was  $+0.297$  ( $SE = 0.045$ ,  $n = 12$ ), which differs significantly from 0 ( $t = 6.66$ ,  $P < 0.001$ ), showing that

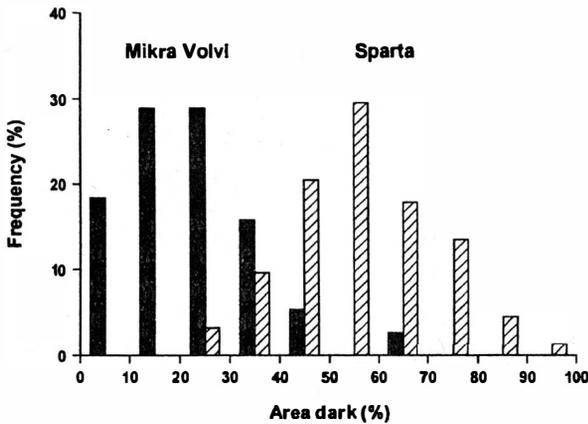


FIG. 2. Individual variation in the area of dark pigmentation on the plastron at the extremes of the cline: Mikra Volvi in northern Greece (solid bars), and Sparta in southern Greece (hatched).

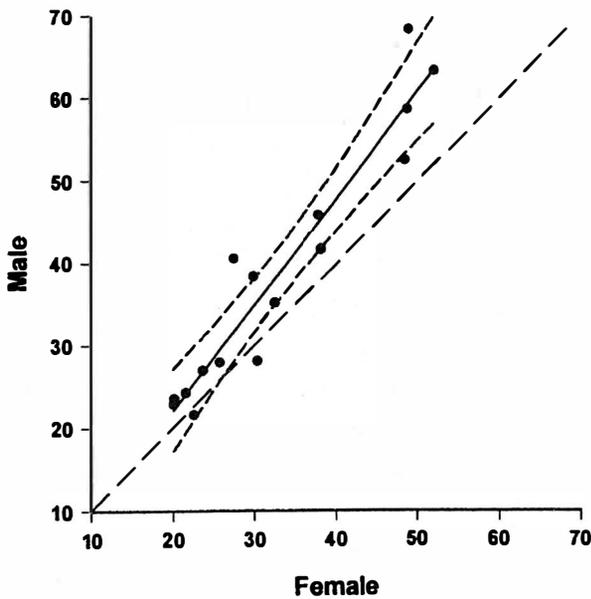


FIG. 3. Sexual differences in the area of dark plastral pigmentation among Greek populations of *T. hermanni*. Each point shows one site, with the fitted regression (unbroken line) and its 95% confidence interval (short dashes). The long dashes show male=female pigmentation.

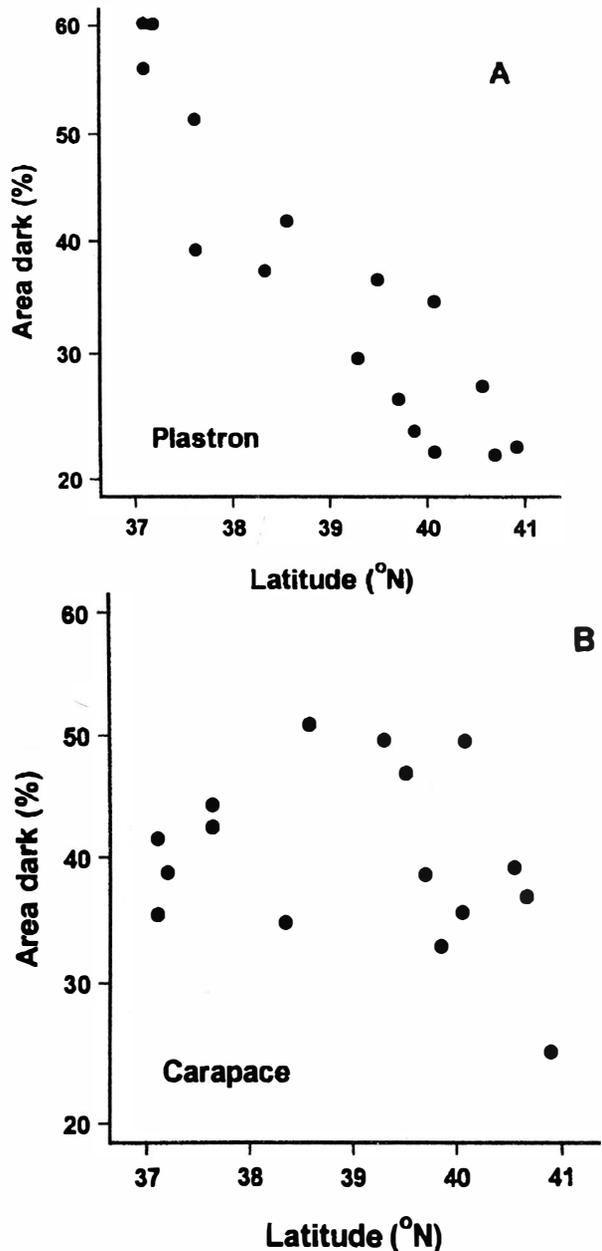


FIG. 4. Latitudinal variation in the area of dark pigmentation on (A) the plastron and (B) the carapace.

TABLE 2. Correlations of dark pigmentation with environmental variation among sites.  $r$  is the correlation coefficient,  $P_r$  is the probability that  $r=0$ , and  $P_b$  is the probability that the coefficient in a multiple regression,  $b=0$ .  $n=16$  sites for each analysis, of arcsine-transformed percentages.

	Area of dark pigmentation (%)					
	Plastron			Carapace		
	$r$	$P_r$	$P_b$	$r$	$P_r$	$P_b$
Latitude	-0.922	<0.001	<0.001	-0.243	0.365	0.386
Longitude	0.026	0.925	0.772	-0.156	0.564	0.597
Altitude	-0.318	0.229	0.087	0.036	0.894	0.814
ET	0.687	0.003	-	0.087	0.749	-

there was a positive association between pigmentation on the upper and lower body surfaces within populations, which was consistent across sites. Nevertheless, the mean value of  $r^2$  was only 11%. Only a small proportion of the within-population variation in the area of dark plastral pigmentation could therefore be explained by the area of dark pigmentation on the carapace.

#### VARIATION AMONG POPULATIONS

The mean area of dark pigmentation varied widely between sites (Table 1) on both plastron (range 22-60%) and carapace (range 25-51%). Table 2 shows an analysis of dark pigmentation in relation to environmental variables. The area of dark pigmentation on the plastron was significantly negatively related to latitude, with a trend over about 400 km between northern and

southern Greece (Fig. 4A). Plastral pigmentation was also significantly correlated with ET, but less strongly than with latitude (Table 2); there was no significant effect of altitude. A multiple regression of the three primary environmental variables showed a highly significant effect of latitude, but not of altitude or longitude (Table 2). (ET was excluded from the multivariate analysis as this was a secondary variable, itself calculated from latitude and altitude; rainfall was excluded for the same reason). In contrast, neither latitude (Fig. 4B) nor any other environmental variable had a significant effect on the area of dark pigmentation on the carapace (Table 2).

#### BODY AND SUBSTRATE TEMPERATURES

Mean  $T_b$  varied significantly between the three sites (Table 3), being lowest at the hot, southern site and

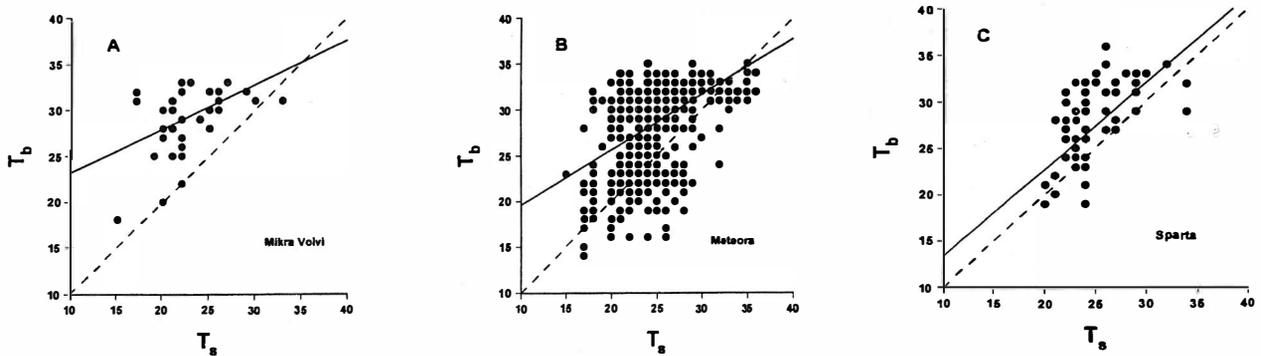


FIG. 5. The relationship between body temperature ( $T_b$ ) and substrate temperature ( $T_s$ ) at three sites in northern (A, Mikra Volvi), central (B, Meteora), and southern Greece (C, Sparta). The dashed line shows  $T_b=T_s$ , and the continuous line shows the regression fit. Note that  $T_b$  is more elevated above  $T_s$  and the slope is lower at Mikra Volvi than at Sparta, with Meteora intermediate in both respects. Regression equations are: (A)  $T_b = 18.5 + 0.474 T_s$  ( $n=42$ ,  $r^2=21.4\%$ ); (B)  $T_b = 13.5 + 0.605 T_s$  ( $n=603$ ,  $r^2=22.5\%$ ); (C)  $T_b = 4.0 + 0.933 T_s$  ( $n=72$ ,  $r^2=41.9\%$ ).

TABLE 3. Body temperature ( $T_b$ ), substrate temperature ( $T_s$ ), and the difference  $T_b-T_s$  at three sites of similar altitude in northern, central, and southern Greece. Data ( $^{\circ}\text{C}$ ) shown  $\pm$ SD or with sample size in parentheses. The  $F$  and  $P$  values are for ANOVAs comparing the sites or the sexes.

Site	$n$	$T_b$	$T_s$	$T_b-T_s$	$T_b-T_s$		$F$	$P$
					Males	Females		
Mikra Volvi	42	29.3 $\pm$ 3.5	22.7 $\pm$ 3.5	6.6 $\pm$ 3.6	6.8 (33)	5.7 (9)	0.67	0.42
Meteora	603	28.5 $\pm$ 4.6	24.8 $\pm$ 3.6	3.7 $\pm$ 4.2	4.0 (316)	3.4 (287)	2.52	0.11
Sparta	72	26.9 $\pm$ 4.6	24.5 $\pm$ 3.2	2.4 $\pm$ 3.5	2.0 (42)	2.9 (30)	1.02	0.32
$F$		4.73	6.71	13.4				
$P$		0.009	0.001	<0.001				

highest at the cool, northern site. Tortoises achieved high  $T_b$  at Mikra Volvi in spite of environmental temperatures being lower there, both in terms of ET (Table 1) and  $T_s$  (Table 3). Body temperature at Mikra Volvi was substantially elevated above  $T_s$ , especially in cool environmental conditions (i.e. at low  $T_s$ ; Fig. 5A). The mean difference  $T_b - T_s$  varied significantly between the three sites (Table 3), being greatest at Mikra Volvi and lowest at Sparta. Mean  $T_b$  exceeded  $T_s$  at all three sites, so that on average heat would be lost from the plastron to the substrate during activity. The difference between  $T_b$  and  $T_s$ , and thus the potential rate of heat loss, increased further north. There were no consistent or significant differences between the sexes in the elevation of  $T_b$  above  $T_s$  (Table 3).

The regression of  $T_b$  on ambient temperature ( $T_a$ ) may be used as a measure of thermoregulation (Huey & Slatkin, 1976); a slope of 1.0 indicates that  $T_b$  depends on ambient temperature, while a slope of 0 indicates that  $T_b$  is independent of ambient temperature. This measure of thermoregulation may sometimes be misleading (Hailey & Coulson, 1996a), but remains useful in comparisons between populations. The regression slope of  $T_b$  on  $T_s$  was 0.474 at Mikra Volvi (Fig. 5A), significantly different from 1.0 (SE=0.144,  $t=3.65$ ,  $P<0.001$ ), showing that  $T_b$  was particularly elevated at lower  $T_s$ . The regression slope was 0.933 at Sparta (Fig. 5C), not significantly different from 1.0 (SE=0.131,  $t=0.51$ ,  $P>0.5$ ). The pattern at Meteora was intermediate between the other two sites in both the mean elevation of  $T_b$  above  $T_s$  (Table 3), and in the slope and intercept of the regression of  $T_b$  on  $T_s$  (Fig. 5B). The regression slope of 0.605 at Meteora was also significantly different from 1.0 (SE=0.046,  $t=8.59$ ,  $P<0.001$ ). The slope of  $T_b$  on  $T_s$  was significantly different from 0 at all three sites:  $P=0.002$  at Mikra Volvi and  $P<0.001$  at Meteora and Sparta. These data therefore show greater thermoregulation in the north, both in terms of the elevation of  $T_b$  above  $T_s$  and the slope of  $T_b$  on  $T_s$ .

## DISCUSSION

### EFFECT OF INCUBATION CONDITIONS?

A cline (a gradient in a measurable character; Huxley, 1938) of plastral pigmentation clearly occurs in *T. hermanni* in Greece along a north-south axis. Variable pigmentation occurs within many other, perhaps most, reptile species. Such variation usually occurs, however, within populations (polymorphism; Forsman & Shine, 1995), between habitats (Gibbons & Lillywhite, 1981), at a microgeographic scale (Thorpe, Black & Malhotra, 1996), or between races or subspecies (Fritz, 1992). We know of no other well-documented example of a cline of pigmentation of a reptile species over a distance of hundreds of kilometres. The first hypothesis to be examined is whether the cline represents a phenotypic response to incubation conditions. Low incubation temperature causes a decrease in the area of dark plastral pigmentation in emydid turtles (Etchberger *et al.*, 1993). A similar ef-

fect might explain the cline of pigmentation in *T. hermanni*, if nest site selection was unable to compensate for differences in climate among populations.

Pigmentation was more strongly correlated with latitude than with ET and was not related to altitude, suggesting that differences in pigmentation were not a purely phenotypic response to incubation temperature. Two other lines of evidence support this conclusion. First, sex is environmentally determined in *T. hermanni* (Eendebak, 1995) as in many other chelonians (Janzen & Paukstis, 1991), with females being produced at higher temperatures. Pigmentation was greater at warmer sites; if this was due to incubation temperature, then females should be more highly pigmented than males. In fact, males had consistently greater plastral pigmentation than females (Fig. 3). Second, *T. hermanni* hatched in captivity show no noticeable effects of incubation temperature (over a wide range, from 24-34°C) on pigmentation (B. T. Eendebak, personal communication). Although it is not possible to rule out some effect of incubation temperature on plastral pigmentation in *T. hermanni*, this will certainly be too small to explain the wide variation of mean pigmentation among field populations (Fig. 4B). The hypothesis that variation of pigmentation between sites is a purely phenotypic effect of incubation temperature can therefore be rejected.

### ADAPTIVE OR RANDOM VARIATION?

Although differences in pigmentation are probably genetic, this still leaves the question of whether they are adaptive or due to chance events. Random differentiation along a cline depends on the appropriate level of genetic exchange (of individuals and genes) between adjacent populations: too high, and the populations become uniform; too low, and a mosaic of local differences is produced rather than a broad geographic trend (Endler, 1977). Home ranges of tortoises are generally small in relation to the scale of the clines observed here: home ranges of *T. hermanni* are of a few hectares (Hailey, 1989), implying movements of a few hundred metres in any direction. Nevertheless, longer movements by a few transient individuals (Kiestler, Schwartz & Schwartz, 1982) could be enough to promote gene flow between populations.

Random differentiation along a cline may occur through secondary contact between populations which have differentiated when separate, or among contiguous populations as a result of genetic drift or recurrent mutation in certain areas (Endler, 1977). Any of these three models could apply to *T. hermanni* in Greece. The distribution of this species is currently continuous over the whole country, as required by the latter two models. Analysis of mitochondrial DNA suggests, however, that animals from the Peloponnese are distinct from those from other parts of Greece (A. C. van der Kuyl, J. T. Dekker, J. Goudsmit, D. Ballasina & R. E. Willemsen, in preparation), so that the first model could also apply.

Random differentiation is usually assumed as a hypothesis of last resort, where selective differences are unknown and cannot be conceived (Endler, 1977). The possibility that the cline in *T. hermanni* is due to random differentiation is therefore provisionally rejected, in favour of adaptation to some environmental variable, although this cannot be disproved.

#### ADAPTATION TO SOIL TYPE OR RAINFALL?

Adaptive differentiation of populations along a cline may occur along a continuous environmental gradient, across an abrupt change in the environment, or as a result of adaptation by populations when allopatric and subsequently merging (Endler, 1977). The cline of dark plastral pigmentation was not steep, but occurred over about 400 km. This pattern suggests that any environmental influence was along a continuous rather than a step gradient (for example, a sharp transition of soil types). The direction of the cline also suggests that soil type is not involved, because the geology of Greece varies along an east-west rather than a north-south axis. The tortoise sites fell in two major geological regions; the western sites 1-10 in the Hellenides (Greek fold-mountains), and the eastern sites 11-16 in the northern crust block (Newbigin, 1943). Geological variation within the western mountains also occurs along an east-west axis perpendicular to the coast (Newbigin, 1943).

Rainfall is related to longitude in Greece ( $P=0.002$ ) and does not vary significantly with latitude ( $P=0.983$ ; Willemsen & Hailey, 1999). Rainfall data were not available for most tortoise sites and, although these could be calculated from longitude, the resulting secondary variable could not be included in the multivariate analysis of pigmentation. Nevertheless, the east-west axis of rainfall shows that this variable is unable to explain the north-south cline of pigmentation.

#### PLEIOTROPIC EFFECT OF CARAPACE PIGMENTATION?

The environmental factor most likely to explain a north-south cline of pigmentation is thermoregulation. The radiation balance (rather than ET) will be particularly important in tortoises because their  $T_b$ 's are not closely related to air temperature (Meek, 1988); light intensity is the most important physical factor in determining heating rates of terrestrial turtles (Boyer, 1965). Colour affects the absorbance of visible radiation (Mount, 1979), and this effect has been shown in comparisons of lizards from different areas (Hutchison & Larimer, 1960). Visible light may make up about half of the total radiant heat load on an animal (Finch, 1972), the remainder being infra-red; body colour may thus have a major effect on thermal balance. Nevertheless, there was no cline of pigmentation on the carapace: the cline of plastral pigmentation is therefore not a pleiotropic effect (i.e. a multiple effect of a single gene) of selection for carapace colour.

#### SELECTIVE ADVANTAGE OF PLASTRON COLOUR

The plastron may be an important route of heat loss from tortoises (Mackay, 1964; Lambert, 1981), by con-

duction/convection or emission of infrared radiation. Most heat exchange through the plastron will be by conduction/convection, especially at rest when the plastron is in contact with the substrate, but this will be independent of colour. Heat exchange through the plastron will also occur by infrared radiation when the body is raised above the ground during activity, by emission from the body and absorption of infra-red re-radiated from the ground. Coleman & Livezey (1968) found small decreases in infrared reflectance (and thus increases in emissivity) of dark compared to light areas of skin in the lizard *Sceloporus occidentalis*. Plastral pigmentation may therefore have a slight effect on the infra-red balance; and even a slight thermal advantage may be important as the colour of the plastron is not under strong selection for camouflage.

The rate of heat loss from the plastron to the substrate depends on the difference between  $T_b$  and  $T_s$ , which was greatest in the north. This heat loss would be minimized by reducing the amount of dark pigmentation on the plastron. Body temperature was much less elevated above  $T_s$  in the south, so that there would be little heat loss or thermal disadvantage from a high level of dark pigmentation. The sexual difference in plastral pigmentation remains unexplained, since there was no consistent sexual difference in the elevation of  $T_b$  above  $T_s$ . It is possible that the sexual difference in pigmentation is not functional, but due to the difference in the shape of the plastron between males and females.

A functional explanation for the cline of dark pigmentation in *T. hermanni* based on thermoregulation would explain why similar clines are absent, or at least uncommon, in other reptiles. Many lizards use changes in skin pigmentation as a means of physiological thermoregulation, with increasing reflectance of both visible and near infra-red radiation at higher body temperature (Bartholomew, 1982). Pigmentation in the scutes of tortoises cannot be changed behaviourally, so that adaptation must occur at the level of the population (producing a cline) rather than the individual. Pigmentation of tortoises is in this respect more similar to that of insects, in which geographical variation of colour for thermoregulation is well-documented, than to other reptiles. For example, dark pigmentation varies within and between species of *Colias* (clouded yellow butterflies) with latitude (Watt, 1968), and has significant effects on their thermoregulation (Watt, 1969).

#### THERMOREGULATION AND LATITUDE

Thermoregulation of *T. hermanni* in northern Greece is similar to the pattern found further north in Yugoslavia (Meek, 1988), with  $T_b$  elevated above ambient temperature, particularly in cool conditions (Fig. 5A) as a result of basking. Thermoregulation at Sparta appears to be more similar to that of the tropical tortoise *Kinixys spekii*, in which there was little elevation of  $T_b$  above ambient temperature and a slope of  $T_b$  on  $T_a$  close to 1.0 (Hailey & Coulson, 1996a). It is likely that the avoidance of critically high  $T_b$  is more important than gaining or conserving heat in southern Greece, similar

to *K. spekii* in which thermoregulation consists of avoiding high  $T_b$  by choice of activity time and microhabitat (Hailey & Coulson, 1996a,b).

Mean  $T_b$  at Sparta was lower than that further north, although data were collected slightly later in spring so that differences in the timing of fieldwork should have produced slightly warmer conditions at Sparta, if anything;  $T_s$  was higher at Sparta than at Mikra Volvi. Low  $T_b$  at Sparta also suggests that thermoregulation there consists of minimizing the risk of overheating, by allowing a wider margin before  $T_b$  reaches critical levels. The low mean  $T_b$  at Sparta is also similar to that of *K. spekii* (27.0°C), which has the lowest  $T_b$  reported for any tortoise species despite occupying a hot tropical environment (Hailey & Coulson, 1996b). It is notable that activity of *Testudo graeca* is apparently limited by high environmental temperature in the far south of Europe; tortoises in southwestern Spain (at about 37°N, the same latitude as Sparta) aestivate in summer (Diaz-Paniagua, Keller & Andreu, 1995).

A question remains over the large area of dark plastral pigmentation in western populations of *T. hermanni* (Guyot & Devaux, 1997). Tortoises in southern France have a similar relation between  $T_b$  and  $T_s$  to that found at Sparta; the slope of  $T_b$  on  $T_s$  is about 1 (Fig. 3b of Pulford, Hailey & Stubbs, 1984), and  $T_b$  is only 1-3°C higher than  $T_s$  (Table 1 of Huot-Daubremont, Grenot & Bradshaw, 1996). These data are consistent with the thermal hypothesis for plastral pigmentation. Nevertheless, it is unclear why thermoregulation in southern France, at a latitude of 43-44°N, is similar to that of Sparta rather than northern Greece or Yugoslavia.

#### IMPLICATIONS FOR CONSERVATION

In conclusion, Greek populations of *T. hermanni* show marked variation of dark plastral pigmentation with latitude, but not longitude or altitude. There was a linear cline over more than 400 km, with the area of dark pigmentation being greatest in southern Greece. The cline is unlikely to be due to a purely phenotypic effect of incubation temperature, to variation with rainfall or geology, or to pleiotropic effect of selection for pigmentation on the carapace. Data on body and substrate temperatures are consistent with the hypothesis that the variation is functional, plastral pigmentation being reduced in northern populations to minimize loss of heat from the body to the substrate during activity. An alternative of random genetic variation causing the cline cannot be ruled out, but either of these explanations involves genetic differences between Greek populations of *T. hermanni*.

Greek populations differ widely in adult body size, which is also likely to be due to genetic differences as size was not related to growth rates (Willemsen & Hailey, 1999). Such differences between populations mean that caution must be exercised over the release of tortoises into the wild (Guyot & Devaux, 1997), such as captive-bred individuals or those seized by customs

(for example Ballasina, 1992). This problem has so far only been considered at the level of the eastern and western subspecies. The differences demonstrated here suggest that even greater caution is needed; tortoises should not be released unless their site of origin is known to within a few kilometres.

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#### REFERENCES

- Ballasina, D. (1992). Report on the repatriation of tortoises to Greece by the Carapax Centre (Italy). *Bull. Brit. Herpetol. Soc.* **40**, 2-4.
- Bartholomew, G. A. (1982). Physiological control of body temperature. In *Biology of the reptilia*, Vol. 12, 167-211. Gans, C. and Pough, F. H. (Eds). London: Academic Press.
- Boyer, D. R. (1965). Ecology of the basking habit in turtles. *Ecology* **46**, 99-118.
- Cloudsley-Thompson, J. L., Constantinou, C. & Butt, D. K. (1985). Carapace coloration and latitudinal distribution in Testudinae. *Bull. Brit. Herpetol. Soc.* **14**, 10-12.
- Coleman, P. R. & Livezey, R. L. (1968). Infrared reflection of the integument of live *Sceloporus occidentalis* Baird & Girard from three habitats. *J. Herpetol.* **1**, 71-75.
- Crisp, M., Cook, L. M. & Hereward, F. V. (1979). Color and heat balance in the lizard *Lacerta dugesii*. *Copeia* **1979**, 250-258.
- Diaz-Paniagua, C., Keller, C. & Andreu, A. C. (1995). Annual variation of activity and daily distances moved in adult spur-thighed tortoises, *Testudo graeca*, in southwestern Spain. *Herpetologica* **51**, 225-233.
- Eendebak, B. T. (1995). Incubation period and sex ratio of Hermann's tortoise, *Testudo hermanni boettgeri*. *Chelonian Conserv. Biol.* **1**, 227-231.
- Endler, J. A. (1977). *Geographic variation, speciation, and clines*. Princeton, New Jersey: Princeton University Press.
- Etchberger, C. R., Ewert, M. A., Phillips, J. B., Nelson, C. E. & Prange, H. D. (1993). Environmental and maternal influences on embryonic pigmentation in a turtle (*Trachemys scripta elegans*). *J. Zool., Lond.* **230**, 529-539.
- Ewert, M. A. (1979). The embryo and its egg: development and natural history. In *Turtles: perspectives and research*, 333-413. Harless, M. and Morlock, H. (Eds). New York: John Wiley.
- Finch, V. A. (1972). Energy exchanges with the environment of two East African antelopes, the eland and the hartebeest. In *Comparative physiology of*

- desert animals* (Symposia of the Zoological Society of London, Vol. 31), 315-326. Maloiy, G. M. O. (Ed.). London: Academic Press.
- Forsman, A. & Shine, R. (1995). The adaptive significance of colour pattern polymorphism in the Australian scincid lizard *Lampropholis delicata*. *Biol. J. Linn. Soc.* **55**, 273-291.
- Fritz, U. (1992). On the intraspecific variation of *Emys orbicularis* (Linnaeus, 1758). 2. Variation in East Europe and redefinition of *Emys orbicularis orbicularis* (Linnaeus, 1758) and *Emys o. hellenica* (Valenciennes, 1832) (Reptilia, Testudines, Emydidae). (In German.) *Zoologische Abhandlungen Staatliches Museum für Tierkunde Dresden* **47**, 37-78.
- Gibbons, J. R. H. & Lillywhite, H. B. (1981). Ecological segregation, color matching, and speciation in lizards of the *Amphibolurus decresii* species complex (Lacertilia, Agamidae). *Ecology* **62**, 1573-1584.
- Gibson, A. R. & Falls, J. B. (1979). Thermal biology of the common garter snake *Thamnophis sirtalis*: 2, the effects of melanism. *Oecologia (Berlin)* **43**, 99-110.
- Guyot, G. & Devaux, B. (1997). Variation in shell morphology and color of Hermann's tortoise, *Testudo hermanni*, in southern Europe. *Chelonian Conserv. Biol.* **2**, 390-395.
- Hailey, A. (1989). How far do animals move? - Routine movements in a tortoise. *Can. J. Zool.* **67**, 208-215.
- Hailey, A., & Coulson, I. M. (1996a). Temperature and the tropical tortoise *Kinixys spekii*: tests of thermoregulation. *J. Zool., Lond.* **240**, 537-549.
- Hailey, A., & Coulson, I. M. (1996b). Temperature and the tropical tortoise *Kinixys spekii*: constraints on activity level and body temperature. *J. Zool., Lond.* **240**, 523-536.
- Huey, R. B. & Slatkin, M. (1976). Cost and benefits of lizard thermoregulation. *Q. Rev. Biol.* **51**, 363-384.
- Huot-Daubremont, C., Grenot, C. & Bradshaw, D. (1996). Temperature regulation in the tortoise *Testudo hermanni*, studied with indwelling probes. *Amphibia-Reptilia* **17**, 91-102.
- Hutchison, V. H. & Larimer, J. L. (1960). Reflectivity of the integuments of some lizards from different habitats. *Ecology* **41**, 199-209.
- Huxley, J. S. (1938). Clines: an auxiliary taxonomic principle. *Nature* **142**, 219-220.
- Janzen, F. J. & Paukstis, G. L. (1991). Environmental sex determination in reptiles: ecology, evolution and experimental design. *Q. Rev. Biol.* **66**, 149-179.
- Kiester, A. R., Schwartz, C. W. & Schwartz, E. R. (1982). Promotion of gene flow by transient individuals in an otherwise sedentary population of box turtles (*Terrapene carolina triunguis*). *Evolution* **36**, 617-619.
- Lambert, M. R. K. (1981). Temperature, activity and field sighting in the Mediterranean spur-thighed or common garden tortoise *Testudo graeca* L. *Biol. Conserv.* **21**, 39-54.
- Lawrence, J. & Wilhoft, D. (1958). Cryptic coloration in lava-dwelling horned lizards. *Copeia* **1958**, 43-44.
- Lewis, T. H. (1949). Dark coloration in the reptiles of the Tularosa malpais. *Copeia* **1949**, 181-184.
- Mackay, R. S. (1964). Galapagos tortoise and marine iguana deep body temperatures measured by radio telemetry. *Nature* **204**, 355-358.
- Meek, R. (1988). The thermal ecology of Hermann's tortoise (*Testudo hermanni*) in summer and autumn in Yugoslavia. *J. Zool., Lond.* **215**, 99-111.
- Meteorological Office. (1996). *Tables of temperature, relative humidity, precipitation and sunshine for the world, Part III: Europe and the Azores*. London: Meteorological Office.
- Mount, L. E. (1979). *Adaptation to thermal environment: man and his productive animals*. London: Edward Arnold.
- Murray, J. D., Deeming, D. C. & Ferguson, M. W. J. (1990). Size-dependent pigmentation-pattern formation in embryos of *Alligator mississippiensis*: time of initiation of pattern generation mechanism. *Proc. R. Soc. (B)* **239**, 279-293.
- Newbigin, M. I. (1943). *Southern Europe (second edition)*. London: Methuen.
- Porter, W. P. & Norris, K. S. (1969). Lizard reflectivity change and its effect on light transmission through body wall. *Science (Washington DC)* **163**, 482-484.
- Pulford, E., Hailey, A. & Stubbs, D. (1984). Thermal relations of *Testudo hermanni robertmertensi* WERMUTH in S. France. *Amphibia-Reptilia* **5**, 37-41.
- Sokal, R. R. & Rohlf, F. J. (1981). *Biometry (second edition)*. San Francisco: W. H. Freeman & Co.
- Stubbs, D., Hailey, A., Pulford, E. & Tyler, W. (1984). Population ecology of European tortoises: review of field techniques. *Amphibia-Reptilia* **5**, 57-68.
- Stuckenberg, B. R. (1969). Effective temperature as an ecological factor in southern Africa. *Zool. Afr.* **4**, 145-197.
- Thorpe, R. S., Black, H. & Malhotra, A. (1996). Matrix correspondence tests on the DNA phylogeny of the Tenerife lacertid elucidate both historical causes and morphological adaptation. *Syst. Biol.* **45**, 335-343.
- Watt, W. B. (1968). Adaptive significance of pigment polymorphism in *Colias* butterflies. I. Variation of melanin pigment in relation to thermoregulation. *Evolution* **22**, 437-458.
- Watt, W. B. (1969). Adaptive significance of pigment polymorphism in *Colias* butterflies. II. Thermoregulation and photo-periodically controlled melanin variation in *Colias eurytheme*. *Proc. Natl Acad. Sci. USA* **63**, 767-774.
- Willemssen, R. E. & Hailey, A. (1989). Status and conservation of tortoises in Greece. *Herpetol. J.* **1**, 315-330.
- Willemssen, R. E. & Hailey, A. (1999). Variation of adult body size of the tortoise *Testudo hermanni* in Greece: proximate and ultimate causes. *J. Zool., Lond.* (in press).